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COMPARATIVE STUDIES WITH LABELED HERBICIDES ON WOODY PLANTS[1]

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INTRODUCTION

LARGE AREAS of range land, partly or almost completely covered with woody plants, constitute one of the greatest opportunities for use of herbicides. There are around 100 million acres of such land in the southwestern United States, and the southeastern and eastern states must have at least an equal area. Add to this a fair portion of the continents of Africa, Asia, South America, and Australia and it seems apparent that the control of brush and woody plants constitutes one of the major remaining frontiers for increasing agricultural production.

Tremendous strides have been made in the use of chemicals in such areas where much testing is currently being done. However, because most of the preliminary testing is done on plots in the field, all of which must be read and interpreted if it is to add to our understanding of woody plant control, it seems desirable to continue basic physiological work on the uptake and distribution of chemicals in such plants. And recent work in which several labeled herbicides have been used comparatively (Crafts and Yamaguchi, 1958)[4] has indicated the value of this method for obtaining a clear picture of what goes on in the intact plant. Only as the behavior of each new chemical is worked out are we able to understand the reasons for its success or failure in the field.

An earlier paper (Leonard and Crafts, 1956) pointed the way to methods for using labeled compounds in such studies. It indicated some of the factors of climate, season, and plant characteristics that determine the amount of a given chemical absorbed and its subsequent distribution. This paper continues the work along a new approach and circumscribes those factors that determine the comparative mobility of different herbicides in woody plants. It does not attempt to describe new field methods. Rather it is pointed at a clearer understanding of the basic responses of woody plants to herbicidal compounds and should provide answers to a number of questions that arise in the mind of every field man who has done comprehensive plot work.

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[4] See "Literature Cited" for citations referred to in text by author and date.

Theoretical Background

Before describing the experimental work under consideration, it seems desirable to discuss the mechanical aspects of solute movement in plants. First, it should be recognized that two tissue systems are responsible for rapid movement of materials in plants—the xylem and the phloem. Second, it is apparent from tracer studies that materials may move from one of these systems to the other, and this movement may take place via the symplast or living protoplasm or via the apoplast or dead cell wall phase. Third, all applications are to the apoplast, and any material that moves in the symplast must first be absorbed by protoplasm.

Concerning xylem transport, it is well agreed that movement in general is from roots to shoots and leaves in the transpiration stream. It is less well agreed, but seems increasingly apparent, that phloem movement is from source (the site of photosynthesis or storage) to sink (the site of growth or storage) in the so-called assimilate stream. Such movement involves concurrent movement of solute (assimilates) and solvent (water) along a gradient of hydrostatic pressure. It does not require that all constituents of the stream move at the same rate, because the stream must pass through very numerous sieve plates in the sieve tubes, and these, by a differential filtering action, may retard movement of large molecules or molecules having specific bonding sites, compared with the solvent water, and small uncharged molecules. And every compound in the assimilate stream would be expected to translocate at somewhat varied rates. Also, the rate of flow of the assimilate stream itself varies with the rate of growth and storage; flow may vary from zero to more than 150 cm per hour. Presumably the rate of movement of a herbicide in the phloem can, at best, approach the rate of the assimilate movement, but is generally reduced by lateral movement leading to absorption and retention by parenchyma or loss to the transpiration stream.

Movement up to and away from the sieve tubes must take place via living parenchyma cells, particularly the border parenchyma of leaves and ray cells of the vascular cylinder. Such symplastic movement is undoubtedly accelerated by protoplasmic streaming.

Movement into and out of the xylem conduits (tracheids and vessels) must take place in the apoplast, because the xylem conduits themselves are dead cells. A mechanism of movement of ions from soil to xylem of roots has been described (Crafts and Broyer, 1938) as being essentially symplastic. Evidence for apoplastic movement of the transpiration stream in the mesophyll of leaves is presented by Yamaguchi and Crafts (1958) and by Strugger (1938).

When a droplet of a tracer is applied to the leaf of a growing plant, the tracer must cross the cuticle and the underlying pectic and cellulose cell walls of the epidermis. Here it may move farther in two ways: It may continue to diffuse along the apoplast and, meeting with the transpiration stream, may be swept toward the periphery of the leaf. Or, if it is absorbed into the living symplast, it may move from cell to cell until it enters the sieve tubes. Here it is swept along with food materials in the assimilate stream to sinks either in the lower stem and roots, or in the outer shoot tips, or both. And some may be retained in the cambium throughout the stem

and root. There is evidence that monuron follows the apoplast route, whereas 2,4-D, ATA, and MH enter and move in the phloem (Crafts and Yamaguchi, 1958). Occasionally a third type of movement takes place. If there is injury to the cuticle so that the aqueous apoplast is exposed, the solution may be sucked directly into the xylem and concentrated along the veins toward the outer edge of the leaf. This is illustrated in Crafts (1956, figure 18).

Treatment on bark, as in the basal spraying of woody plants, presents some of the same problems. For downward movement to roots to occur, the chemical must traverse the corky outer bark layers; it must be absorbed into living cells, it must move via the symplast to the phloem of the inner bark, and here it must be released into sieve tubes. To move upward in the xylem, it must penetrate the outer layers, diffuse along the apoplast across phloem and cambium, and finally it must enter the xylem conduits and move in the transpiration stream.

Apparently solutes in either of these main channels of movement may be absorbed into ray parenchyma cells and move laterally. Most past experience would seem to indicate that such materials tend to remain in the symplast, where they provide for the normal nutrition of the plant. Examples are carbohydrates that may move from phloem via rays to xylem parenchyma and pith, where they are stored as starch. Potassium moving in the transpiration stream may be absorbed by ray parenchyma and moved laterally to cambium and phloem (Stout and Hoagland, 1939).

Clor showed in 1950 that 2,4-D applied to a leaf of cotton would move down into the roots and leak into the ambient culture solution (Crafts, 1956). Many other compounds have since been shown to move from roots to the culture medium. Meanwhile comparative studies with labeled tracers have shown that some compounds, notably MH, will move from phloem to xylem and hence will circulate in plants (Crafts and Yamaguchi, 1958).

There is accumulating evidence that different molecules are handled differently by different cells and tissues of a plant. For example, the phenoxy compounds seem to enter and move in the symplast, and they seem to be immobilized in living parenchyma tissues, as are food reserves. This may place a definite limitation on the extent of their movement from a given source.

Aminotriazole may enter the symplast and move readily in the phloem; it is much less restricted in distribution by accumulation in parenchyma than 2,4-D. Maleic hydrazide seems freely mobile in the symplast and in the sieve tubes. There is little tendency for it to be accumulated in living parenchyma along the transport route. More than other compounds, it is subject to continued redistribution, with a tendency toward piling up in sinks for assimilates. Urea moves rather freely, but in green tissues in the light it may be hydrolyzed and the CO_2* synthesized to sugars.

Monuron applied to a leaf moves only from the treated spot toward the periphery of the leaf (Haun and Peterson, 1954). Applied to roots through culture solution or through the soil, it readily enters the xylem and moves upward in the transpiration stream (Minshall, 1954).

With these various relationships in mind, the present study was conducted to obtain a clearer picture of herbicidal movement in woody plants.

METHODS AND MATERIALS

Whereas the earlier work involved application of 2,4-D* to leaves and subsequent autographing of bark, shoots, et cetera above and below the point of application to find its distribution, the present study was made by applying a number of labeled compounds to the inner bark of the trunk of trees. In preparation, several trees of 1- to 2-inch diameters were selected; and at a convenient height the region for treatment was marked on each. At the time of treatment a series of six spots about ½ inch apart was prepared by cutting away the outer bark and exposing the current season's functional phloem. The active phloem was recognizable from February through August by its translucency.

TABLE 1

CHEMICALS AND THEIR MOLECULAR WEIGHTS AND CONCENTRATIONS USED IN TREATING WOODY PLANTS

Name of chemical	MW	conc.—ppm
2,4-D	221.0	2,500
2,4,5-T	255.5	2,890
ATA	84.5	956
MH	112.0	1,267
Urea	60.06	679
Monuron	198.0	2,240

A small lanolin cup was built below and around each exposed spot to hold the treatment solution. The usual dose was 20 lambdas having 0.1 microcurie activity.

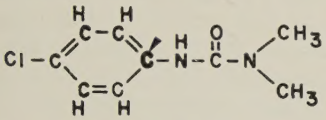
After the outer bark was removed and the inner phloem exposed, there remained the inner phloem and cambium comprising about a half millimeter of tissue between the treatment and the xylem. By removing the cortex, resistance to penetration was reduced to a minimum. By using tree trunks with a diameter of 1 to 2 inches, it has been simple not only to compare the movement of six chemicals in one plant, but also to compare them in the phloem and the xylem systems of a single plant, where neither downward nor upward movement needs be limited. Though the toxicity of the chemicals to tissue at the treatment spot cannot be denied, the treatment time has been only one day, and downward movement of the compounds never appeared to be limited because of toxicity.

Three tree species were used, manzanita (*Arctostaphylos manzanita*), an evergreen; toyon (*Photinia arbutifolia*), an evergreen; and buckeye (*Aesculus californica*), a deciduous species. The location was on the banks of Putah Creek about 30 miles west of Sacramento.

In February and March of 1956, four C^{14}-labeled compounds were used: 2,4-D, maleic hydrazide, aminotriazole, and urea. In April, 2,4,5-T and monuron were added. The solvent of the treatment solution was 50 per cent ethyl alcohol plus 0.1 per cent Tween 20. The specific activity of the radioactive chemicals was standarized at 0.44 mc per mM and the concentration was adjusted so that 20 lambdas contained 0.10 μc. This has a mM concentration of 11.3 per liter. Table 1 shows the molecular weights and

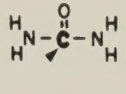
concentrations in parts per million. Text figure 1 shows the molecular configuration of the tracer molecules used and the position of the C^{14} label.

Each month one tree of each species was treated, using all of the chemicals. Time of treatment was between 1 P.M. and 4 P.M. Treatment time was approximately 24 hours. During the early summer some trouble was experienced with the lanolin running. It was found that the lanolin could be thickened by adding granular starch until the viscosity was great enough to resist the melting. Shading the treated areas also helped. After applying the solution droplets, the spots were covered with cellophane to prevent loss or radio-contamination.

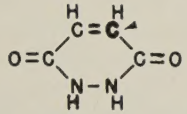


MONURON

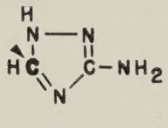
3-(p-CHLOROPHENYL)-1,1-DIMETHYLUREA



UREA

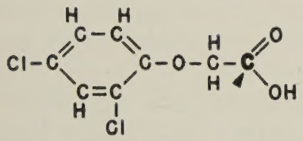


MALEIC HYDRAZIDE



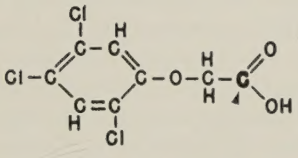
AMITROL

3 AMINO-1,2,4-TRIAZOLE



2,4-D

2,4-DICHLOROPHENOXYACETIC ACID



2,4,5-T

2,4,5-TRICHLOROPHENOXYACETIC ACID

Text figure 1. Molecular configuration of tracer molecules used and position of C^{14}.

Collection of Samples

At the time of collection the lanolin rings were wiped off until the treated spots were clean. Then a ring of bark 4 inches wide and centered at the treated level was separated from the trunk, and rings 2 inches wide from 6- and 12-inch levels above and below the treatment were taken—a total of five bark samples. With manzanita, the bark was cut through at the top and bottom of the desired sample and then split longitudinally at the back of the trunk, away from the treatment spots. Then a corner was pried up and pulled slowly to peel the bark sample. With toyon and buckeye, whose bark was considerably thicker, first the outer half of the bark was cut away and then the bark samples were taken as in manzanita.

All of the material was open-air dried. The rings of bark were allowed to dry momentarily until the inner face was no longer watery. Then they were flattened and held against a piece of board with a square of heavy-gauge ½-inch mesh hardware cloth while they dried. A sheet of paper was placed between the board and the bark samples; the piece of hardware cloth

was rolled into a slight curve to obtain a light springiness when its edges were held against the board. Then the edges were clamped together with large, jaw-type stationery clips (Hunt clip No. 3).

The dried samples were slightly remoistened in a humidity chamber, then flattened in a press, trimmed to uniform size and thickness, glued down to lithograph or filter paper, and autographed on Kodak no-screen X-ray safety film.

During the season when the bark would not peel, the trunks were sampled as indicated in figure 1. Following this scheme, autographs could be made at the level of treatment and ½, 1, 1½, 6 and 12 inches above and below the treatment level.

The cross-sectional samples were always cut on the evening of the day of collection. The trunk sample brought in from the field was held in a vise and the sections were cut with a 10-point cross-cut carpenter's saw. The ⅜-inch thick sections were dried in the air for 10 to 12 hours and then planed smooth with a sharp jack plane on the side to be autographed. They were then dried for 24 hours, mounted on paper, and exposed on film.

RESULTS

Trunk Treatments

The following results are based on autoradiographs of samples collected at short distances, up to a foot, from the treated spot. See for example figures 3, 4, 5, and 6. The amount and distance of movement have been interpreted in terms of bar graphs (fig. 2*a*, *b*, *c*). One must remember the limitation inherent in an experiment of this sort, in which the labeled solution was applied directly to the active phloem of the tree trunk. During the summer months the rapid uptake of the applied solution allowed only a limited time for absorption by the phloem. Under these conditions even the phenoxyacetic acids, the most readily absorbed by the symplast, were largely swept upward in the transpiration stream. Where neither upward nor downward movement showed and the treatment spot was light, undoubtedly the labeled chemical moved out without leaving a trace, especially in the case of urea and MH applied to toyon and buckeye and in the case of monuron applied to manzanita and buckeye in the spring. Therefore, the autoradiographs show only the amount absorbed along the path of translocation, but not the amount carried away by the transpiration stream. The more mobile compounds, with a low tendency for absorption and retention, produced light autoradiographs from the bark and wood samples, and these images represent a picture only of the absorbed portion of the chemical. Therefore, the term "tracing" is preferred over "translocation" or "movement."

On the other hand, where absorption was high and the direction of movement unquestioned, as, for example, in the case of the phenoxyacetic acids, the autoradiographs clearly indicate the actual extent of translocation and the term "translocation" or "movement" is preferred.

2,4-D and 2,4,5-T. The movement of these compounds was closely similar. They were distinctive from the other four in their downward translocatability. They showed downward translocation in March, April, and May in the buckeye; in March, April, May, and June in the manzanita, and June

through August in the toyon. In March and April the movement was mostly or entirely downward in manzanita (fig. 3) and buckeye. From May on into the summer months the downward movement was gradually reduced (fig. 2*a*) and the upward movement became prominent, especially in manzanita (fig. 4) and buckeye. Then, in the later summer, fall, and winter months, all movement in these two species was brought down nearly to a standstill, due to water stress. The cycle then repeats again with downward movement in early spring.

Seasonal trends were the same in the toyon, but some upward movement occurred throughout the year. Toyon, unlike manzanita and buckeye, grew at the bottom of the canyon, where water was more readily available and vigorous root growth could have continued until later in the season, permitting downward movement through the summer months and upward movement even through the fall months.

The annual flush of growth of both manzanita and toyon was completed by mid-May. The annual flush of growth of buckeye was completed by mid-March. In figure 2 the annual growth period is indicated for reference to the period of maximum downward movement of the phenoxyacetic acids.

ATA. Aminotriazole movement downward was generally less than that of the phenoxyacetic acids and also less consistent. We use "movement" rather than "tracing" with ATA because it was highly absorbed by the xylem. Movement was most extensive in manzanita and covered a period from January to July, a wider span of months than for the phenoxyacetic acids, but the amount moved was less. Downward movement during these months was equal to or somewhat less than upward movement. From August through December the movement was essentially upward.

In toyon and buckeye the downward movement of aminotriazole was a quarter or less than that of the phenoxyacetic acids. Most of it occurred in February and April samples, with toyon, and only in the June samples, with buckeye.

Upward movement of ATA in these two species was also less than that of the phenoxyacetic acids, and it appears as though a high degree of absorption in the xylem (fig. 5*a*, *b*, *c*) within 1½ inches above the treated spot could have limited the amount free to move farther up. From the fact that a greater proportion of ATA than of 2,4-D moved upward, we assume that ATA is more subject than 2,4-D to movement in the apoplast. Though more mobile in this sense, it is not so in the sense that it is highly absorbed in the xylem. The xylem of all three species shows a similarly high degree of absorption of ATA.

MH and urea. These two compounds showed closely similar tracings. They showed downward tracing of a foot or more only in manzanita. It occurred in January and February and again in May. Upward tracing was even more erratic. A point to note with regard to limited and erratic tracing is the low intensity of tracing at and near the treatment spot. In this case about half of the treated spots themselves traced lightly, indicating very low retention and free mobility. In the case of the phenoxyacetic acids and ATA the treated spots invariably showed intense images.

In toyon and buckeye all of the treated spots of both MH and urea were light in the autoradiograph and, except for a light tracing in toyon extending

up 1½ inches or more in the vascular tissue, there was no upward or downward tracing. Apparently these compounds were not absorbed and retained under the conditions of the experiment and were almost completely carried away.

Nevertheless, one clue was discovered in the cross-sectional samples of the treatment in January, at which time the rate of movement of the aqueous phase in the stem was probably at its minimum. In toyon there was a light tracing of both MH and urea at the outer surface of the bark, up to 6 inches or more above the treated spot (fig. 6). There was no tracing of these compounds in the inner regions of bark or in the xylem. In the buckeye that was without leaves from August and had an even lower rate of movement in the transpiration stream, there showed a more prominent tracing of both compounds at the outer surface of the bark for a distance of 1½ inches upward. No tracing occurred in the inner bark or wood. In the case of MH there was a dark tracing of the treated spot. January treatment of buckeye was the only one showing a dark tracing of the spot treated with MH. This was possible only because the rate of movement of the aqueous phase in the stem of this deciduous plant during January was nearly at a standstill. In spite of the fact that the pattern of distribution found in January is no indication of the pattern of movement and distribution during other months, in this particular case it definitely demonstrates the extreme mobility of MH and urea in the trunks of toyon and buckeye, and indicates that the mobility in plant tissue is probably an expression of the lack of absorption.

Monuron. The chemicals considered thus far showed a certain mobility relative to one another, and this relationship was held in all three species: the 2,4-D, and 2,4,5-T group, the least mobile, with the most downward movement; ATA, of intermediate mobility, with a little less downward movement; and the MH and urea group, the most mobile, with the least downward tracing. Upward tracings were not so clear-cut. The difference between the three groups was large and distinctive and can be used here as a basis for comparison of the mobility of monuron.

Monuron has generally exhibited a degree of mobility outside of this graded series in herbaceous plants (Crafts and Yamaguchi, 1958). In these woody species monuron exhibited a more restricted movement; it was within the range of comparison with that of MH and urea. In manzanita and buckeye the tracing of even the treated spot was light until midsummer. Both upward and downward tracing were more meager and lighter than that of MH and urea in the manzanita, but a little more extensive and darker than that of MH and urea in the buckeye. From midsummer through winter the treated spots traced dark in these two species, but very little tracing extended above or below the treated spots.

In toyon there was a downward tracing of 1½ inches or more in May and June and again in December and January. The treated spots generally traced with medium intensity. Also a light upward tracing of 6 or 12 inches was shown for different months of the year. This was an unusual case of greater absorption and more intense tracing of monuron than of MH and urea. No explanation is apparent and it may be species peculiarity.

The use of tree trunks provides a view of the movement of herbicide chemicals under conditions of rapid movement in the transpiration stream,

of moisture stress, and of small amounts of chemicals applied to a large plant body. Movement and distribution patterns of these chemicals usually recognized in herbaceous materials were greatly accentuated. At times the more mobile compounds had almost completely moved out of the treated area.

Branch and Small Plant Treatments

Toyon. To obtain a better picture of the more mobile compounds we resorted to sprigs of trees and to very small trees and autographed the entire tree or the entire sprig. In this manner movement of the labeled compounds was reduced and contained within the specimen.

From a group of toyon seedlings started in the greenhouse in the spring, 13 uniform trees about 1½ feet tall were selected. This experiment was conducted in October. The young toyon plants in the greenhouse were divided into two groups. In one group the plants were left intact and the treatment solution, 60 microliters, was applied in a small cup of aluminum foil shaped around the stem 8 to 8½ inches from the stem tip. Good seal was achieved with lanolin-starch paste (lanolin stiffened with granular starch). In the other group the only variable was the removal of about half of the thickness of the bark in a band about ⅛ inch around the stem at the treatment level.[5] Treatments have been carefully restricted to the internode region. Treatment time was one day. The treated spot was cleaned with tissue paper and wrapped with cellophane tape to prevent radioactive contamination. The plants were harvested an inch below the treated level with a long tapered cut for autographing any downward movement in the stem. Two inches above the treated spot another long tapered cut was made for autographing the vascular tissue above the treated level. The specimens were air-dried, as were the bark samples.

The autographs showed one consistent contrast between this supplementary experiment and the main experiment, that of predominance of upward movement of the chemicals. The young trees were growing rapidly and they might be compared with the field toyon trees of April, when their annual flush of growth was in progress. In both cases 2,4-D showed movement both upward and downward. Figure 7 shows the uptake and distribution of 2,4-D from an application to an intact spot in the internode and to a "cut" spot in the internode. 2,4,5-T exhibited a distribution pattern similar to that of 2,4-D, except that it showed only upward movement in the young toyon trees of October as well as in the trunks of field toyon trees of April.

With regard to the other chemicals, ATA, MH, urea, and monuron, the movement was almost entirely upward and into the leaves. A notable point about monuron was that the very young leaves showed no tracing at all (fig. 8) while all others did. This is evidence of movement in the transpiration stream and lack of movement in the assimilate stream. All other chemicals showed up in the mature leaves as well as in these very young leaves, indicating a certain amount of transfer from the transpiration stream to the assimilate stream.

The distribution pattern of the chemicals captured in the leaves also provides us with some characteristic qualities of the mobility of these com-

[5] These will be referred to as the "cut" in contrast to the intact condition at the application spot.

pounds. As with applications to the tree trunk, 2,4-D and 2,4,5-T traced dark in the vascular tissues. They marked the veins of the leaves with decreasing intensity from the base to the tip of the leaf blade, leaving the interveinal areas relatively light. The other four compounds exhibited much more uniform distribution in the leaf blade, indicating far greater mobility in the transpiration stream, or possibly a lower degree of absorption. ATA and monuron exhibited somewhat more uniform distribution in the leaf blades than urea and MH. Urea produced uniform tracing of the interveinal areas of the leaf as did MH, but it also traced the veins and the stem. This may be a redistribution of some compound formed from urea decomposition.

The matter of differences in result due to the "cut" at the application spot is one of absorption and penetration through the epidermis and cortical tissue. Without the "cut" there was absorption of all the chemicals as shown in the autographs, but with the "cut" the amount carried to the leaves was more than doubled, except in the case of ATA. ATA, MH, and 2,4-D were well absorbed and distributed without being "cut" at the application spot.

In September a similar experiment was conducted with field material. Seven sprigs growing on trees were selected for uniformity and for the presence of this year's as well as the previous year's foliage. In the region of the previous year's foliage, the outer bark was "cut" and a band of about ⅛ inch around the stem removed, without any attempt to expose the active phloem. The same six chemicals were used and the treatments were the same as with the young toyon trees. However, instead of long diagonal cuts, thin cross sections were taken at 2-inch intervals above and below the treated level. The treatment time was also 24 hours.

In contrast to the small young trees, these showed lack of downward movement of even the phenoxyacetic acids. Also, upward movement was very much limited. The toyon sprigs in the field were "cut" at the application spot, yet only ATA exhibited a fair amount of movement into the leaves (fig. 9). In the order of increasing uniformity or freeness of distribution in the leaf blade, they are 2,4-D and 2,4,5-T; monuron and urea; ATA and MH. ATA and MH gave the most uniform tracing of the toyon leaves and undoubtedly they are the most mobile, at least in the leaves of toyon. However, the greatest mobility in the trunk had been indicated for urea and MH, but not for ATA, whose limited movement in the trunk appeared to be caused by a unique case of absorption in the xylem. As shown in figure 5*a*, *b* and *c*, ATA exhibited a greater degree of absorption in the xylem of all three species than any other chemical.

The fact that the compounds showed wider differences of distribution in the sprigs of field trees than in the greenhouse trees is believed to be due to greater water stress and consequent low rate of flow of the transpiration stream.

Stem cross sections taken at 2-inch intervals above and below the treatment level revealed only upward movement of the labeled chemicals. This correlates well with the periods of upward tracing, presented in the bar-graphs of figure 2. In these cross sections, as in those of the December treatment of the main experiment, urea showed an accumulation at the surface of the bark.

Manzanita. To study the nature of the mobility especially of monuron in

manzanita an experiment was conducted with small manzanita plants. In July, 37 plants of *Arctostaphlos viscida*, a species different from, but similar to, the one in the main experiment were selected. Plants about 8 inches tall and about three years old were growing in a burned-over area at Rescue, southeast of Sacramento. The selected plants all had some young leaves, but the ground was very dry and certainly the growth rate was very slow. The plants were divided into three groups. There were 2- and 24-hour treatment time groups in which thin longitudinal strips were removed from the outer bark at three or four places around the main stem at ground level. This was done to break the penetration barrier at the outer bark. No attempt was made to expose the active phloem. These will be referred to as the "cut" groups, in contrast to the intact group. At this level a cup was formed around the stem with aluminum foil and lanolin-starch paste. In another 24-hour treatment time group the outer bark was left intact and the cup was formed around the stem at the same level. Sixty microliters of the labeled solution were applied to each cup. The same six chemicals were used. Two plants per experimental variable were used.

After the treatment period the cups were removed, the lanolin-starch paste was wiped off, and the treated region was wrapped with cellophane tape. The plants were dug up to retain some of the roots. They were air-dried—as were the bark samples—mounted, and each entire plant was autoradiographed.

In the two-hour treatment time group ("cut") the solution was all absorbed and appeared to be distributed mostly in the stem above the application level, as attested by the autoradiographic image of the lower parts of the stems. In the case of 2,4-D, ATA, MH, and urea, the longitudinal cuts through the stem 2 inches above the application level showed large quantities in the xylem and the inner bark regions. Monuron, however, traced the bark upward intensely 1½ inches. With 2,4,5-T there was little evidence of movement in two hours. In some instances (2,4-D and ATA) the veins of a few lower leaves showed up lightly and in the case of MH some of the upper leaves showed up as light images without any greater intensity along the veins.

Downward movement of a few inches was noted for MH, urea, and 2,4,5-T, with the two-hour treatment time.

The autoradiographs of the 24-hour groups, both intact and "cut," showed a predominating upward movement of the labeled compounds. The intact 24-hour group showed limited uptake and distribution, no more extensive than in the "cut" two-hour group, except in the case of monuron.

Monuron exhibited the same pattern of distribution in the 24-hour treatment, whether the application spot was intact or "cut." A large amount of absorption and movement from an application to intact stem characterized this compound. The outer bark of the manzanita was apparently not a barrier to monuron penetration. Figure 10 shows an autograph of a 24-hour treatment with monuron applied to the intact stem. With monuron the stem showed up very intense for a few inches above the treated spot, indicating a rather large lateral movement toward the outer bark. It appears to be a characteristic of the more mobile compounds when the transpiration stream is moving quite slowly. Because of the large lateral movement, the movement

into the top leaves was not so extensive in 24 hours as it was with MH, whose movement in the stem was apparently more restricted to the vascular tissue.

In the "cut" 24-hour group the appearance of the isotopes in leaves varied from all to none in the following order: MH, ATA, monuron, 2,4,5-T, 2,4-D, and urea. Urea again showed the same lateral movement exhibited by monuron, with an intense image of the stem and bark for a few inches above the treated level. The upward movement into the leaves, being a result of a number of factors, does not necessarily indicate all of the aspects of mobility. An autoradiograph of the distribution of MH is shown in figure 11; note the thorough distribution in the foliage and the light tracing of the stem. This is the opposite of the conditions found with monuron (fig. 10), in which the stems and lower leaves showed intense tracings and the upper leaves light. Note also a root with very intense tracing and a few small roots with very light tracing. Except in this instance, the movement of the chemicals into the roots was not very different from that observed for the "cut" two-hour group—namely, a few inches of the root showed a light tracing with 2,4-D, 2,4,5-T, ATA, MH, and urea, but no tracing with monuron.

In these translocation experiments with woody species, a downward movement of the labeled compound was assumed to occur in the phloem. Actual demonstration of movement of mobile tracers in phloem is difficult (Biddulph, 1956).

Willow. In the following experiments we have manipulated the bark of the willow in various ways to obtain good circumstantial evidence for phloem movement. Four experiments were conducted in August. In the first, four willow plants having stem diameters of ¾ inch were used, one each for 2,4-D, ATA, MH, and monuron. On one side the bark was left intact except at the treatment spot where the outer bark was cut away to expose the active phloem. Usually an area of about a half centimeter across was exposed. A ring of lanolin-starch paste was built up around the exposed area. Ten microliters of the labeled solution were applied per treatment. These four were the control treatments. The experimental treatments were placed on the opposite side of the same four stems and at the same level. An inch above and below the treatment spot a band of bark was removed, exposing an area of xylem about ⅛ by ⅜ inch. In essence this manipulation isolated the phloem longitudinally in the treatment area and the application was essentially an application to the xylem.

Treatment time was one day. Longitudinal strips of the bark and of the xylem were removed, dried, mounted, and autoradiographed. The outer half of the bark was first peeled off and then the inner bark was removed with the least amount of flexing. A thin strip of xylem was also collected from the same region. The collected strips were allowed to dry. Later they were slightly softened in a humidity chamber, then flattened, pressed, mounted, and autoradiographed.

The autoradiographs from the control treatment (fig. 12) showed both upward and downward tracing of 2,4-D, ATA, and MH. Monuron exhibited upward tracing only. Results of the experimental treatment showed upward tracing only of these chemicals (fig. 13). The results speak for upward movement in the xylem only and downward movement in the phloem only, in spite of the presence of the chemicals in both the bark and the wood.

In the next willow experiment four plants having stem diameters of ¾ inch were used to study the requirement of longitudinal phloem continuity for downward translocation. On one side of the stem, phloem continuity was broken by lifting a strip of bark over a distance of 3 or 4 inches and cutting the bark strip loose at the bottom. The treatment spot was located 1 to 2 inches below this break in the phloem continuity. On the other side the lifted strip of bark was severed across the top and the treatment spot was located 1½ to 2 inches above the cut. The treatment time and the handling of the bark and wood strips were the same as in the first willow experiment. The results clearly indicate a pressurized mass flow system in the phloem. Where phloem continuity was broken above the treatment spot, downward tracing directly below the cut was inhibited, but there was some downward movement from lateral transfer, clearly shown with 2,4-D. When the phloem continuity was broken below the treatment spot there was downward translocation to the break. This experiment indicates that translocation in the phloem is not a diffusional type of movement, from cell to cell, but a closed system requiring a pressure head and a sink.

The third experiment used four willow plants, with ¾-inch stem diameters, to test movement of the four chemicals in the phloem separated from the xylem. This separation was effected by making two longitudinal parallel cuts in the bark, ⅜ inch apart and 6 inches long, and very gently prying the strip loose from the xylem. Slightly curved stems were used and the bark was loosened, not on the inside or the outside of the bent, but on the side. A thin strip of polyethylene material was slipped in between the phloem and the xylem. The bark was pressed back in place and wrapped around to prevent undue loss of moisture.

Application of the solutions was made an inch above the lifted region so that at least the phase of absorption and the beginning of translocation would be comparable to the control in the first willow experiment. The results showed downward movement in the phloem without a definite trace in the xylem. Especially 2,4-D, because of absorption, traced well (fig. 14). The amount of downward movement was reduced to less than half by lifting the bark; however, the downward movement was not stopped. The experiment demonstrates that downward movement can occur in the phloem, independent of the xylem. (Compare Stout and Hoagland, 1939.)

The fourth and the last willow experiment conducted in early August was designed to test the movement of these chemicals from the lifted region of the bark across the torn cells and into the xylem during the translocation period. Four curved willow branches 1½ to 3 inches in diameter were selected. Control treatment was put on one side. The experimental treatment was placed near and parallel to the control treatment. Two longitudinal parallel cuts through the bark and ⅜ inch apart were made. The ⅜-inch strip was gently pried and loosened from the xylem over a distance of about 4 inches. Immediately this region was completely and firmly wrapped with masking tape. Then the treatment spots were located on the loosened strip of bark an inch from the top. In the control treatment the bark was not lifted. In both cases the outer bark at the treatment spot was removed, leaving ¼ to ½ mm layer of active phloem. Application of the solution was made in the same manner as in the other willow experiments.

The autographs (fig. 15) show little difference between the control and the experimental treatments. Here, possibly because of the greater rigidity of the thicker bark, loosening of the bark from the xylem did not affect the movement and absorption of the chemicals in the phloem. In fact with both monuron and ATA there seemed to be no difference in movement either in the phloem or xylem brought about by loosening the phloem from the xylem; apparently these chemicals moved into the xylem equally well through torn cambial tissues as well as intact. MH and 2,4-D movement across the torn cambial tissue and into the xylem was less than half that of such movement in the control treatment. The inhibition of movement across the torn tissue is particularly significant with respect to the concept of 2,4-D movement through the symplast rather than through the apoplast. Note that in the case of 2,4-D the tracing of the xylem was relatively light and uniform above and throughout the loosened region, and heavy in the intact region below, proving that translocation is in the phloem. In the intact regions 2,4-D traced equally well in the xylem and phloem, as was the case in the main experiment. In this region it appears that 2,4-D moved from the phloem into the xylem by way of the symplast and was then released into the transpiration stream. In the second willow experiment it was found that the chemicals can move equally well from the xylem to the phloem and the tracing of the phloem and the xylem directly underneath has been the same whether the original movement was in the phloem or in the xylem.

DISCUSSION

Application of the labeled compounds directly to the active phloem in the trunk of trees made it possible to study their movement throughout the year, evergreen and deciduous species alike. The amount of downward movement was much less than would be expected from foliage application, but in foliage application the chemical is applied at the end of the transpiration stream and retained there where time permits gradual movement of the chemical into the sieve tubes. From application to the trunk the absorbed compounds are immediately subject to movement in three different manners: (1) upward in the transpiration stream (aqueous continuity in the apoplast); (2) downward by way of the phloem (symplastic movement); and (3) locally (accumulation). It would be a fair assumption (Crafts and Yamaguchi, 1958) that the more polar compounds by-passed the symplast and went directly to the transpiration stream; that the more lipophilic ones were absorbed by the symplast.

The fact that the more mobile compounds were carried away by the transpiration stream and that only the less mobile ones were phloem translocated does not necessarily mean that ATA, MH, and urea are poorly translocated by manzanita, toyon, and buckeye. Rather, the experiment points out the relative mobility of these compounds through the apoplast and in the final analysis, application is to the apoplast. Relative mobility is an expression of the relative lack of retention by the tissues penetrated. The causes of retention may be various. For example, the chlorophenoxy compounds are unique among these six chemicals in that they were readily absorbed and retained by the symplast under the conditions of these experiments. This absorption is not necessarily metabolic; it can result from the physical-

chemical properties of the molecules, which may be sorbed to protein (Butts and Fang, 1957). Dormant potato tuber tissues as well as active leaf tissues absorb the phenoxyacetic acids in large quantities. Autographs of labeled phenoxyacetic acids are readily differentiated from those of a number of other compounds by their high degree of absorption, regardless of plant species or plant tissues. Here lies the secret of the killing pattern of 2,4-D; it coincides with its distribution pattern. This pattern is one of symplastic absorption and retention in the treated region and along the path of translocation. Extensive downward translocation apparently occurs only when the linear rate of movement of the assimilate stream is high in relation to the absorption rate by living parenchyma.

The absorption of ATA was different in that it was strongly absorbed and retained by the xylem. All three species showed similar retention of ATA in the xylem. Perhaps this retention acts as storage, permitting continued redistribution until the growing points are reached. At any rate the downward translocation of ATA is generally not as limited as that of 2,4-D. Kills of perennials with very extensive underground parts have been obtained with ATA—for example, nutgrass (Anderson, 1958), cattail, and also woody species such as wild blackberry, poison oak, and several oaks (Leonard, 1958).

Leonard (1958) has indicated that the optimum treatment period with ATA is later in the season than for 2,4-D. This indicates a longer period available for effective treatment. The important point here is that while the period of optimum root kill with 2,4-D is correlated with the period of most active movement of the assimilate stream, that of ATA is apparently correlated with periods of low transpiration accompanied by slow downward movement of the assimilate stream. The rate of downward translocation need not be high, but a rapid transpiration stream appears to reduce downward movement of ATA much more than of 2,4-D. ATA has been shown to be subject to movement by the transpiration stream in the treated leaf (Yamaguchi and Crafts, 1958) and in the tree trunk where application of the chemical was made to the active phloem. Therefore, ATA requires foliage application for its movement into the sieve tubes whereas 2,4-D will enter the phloem conduits from the leaf or from application along the stem.

Some of the important characteristics of optimum translocatability are held by ATA. On one hand, 2,4-D, with highly lipophilic properties, exhibits a high degree of symplastic absorption and retention and hence a limited translocatability. On the other hand, MH, with highly polar properties, exhibits a very low degree of symplastic absorption and retention and an excessive mobility, to the extent of free leakage from sieve tubes to vessels. In between the two is ATA, which has the proper balance of various physical and chemical properties for the greatest downward translocation. Efficiency in translocation is increased by cutting out the various losses en route. Consequently ATA accumulates at the growing tips and at intercalary meristems and these meristematic regions are killed first, at least in small plants. A large part of the success of ATA as a herbicide must rest on its translocatability and consequent accumulation at the growing regions.

The usefulness of ATA covers certain smaller woody species, but appears to have a few limitations in coping with larger species. ATA, because of

its low toxicity, can be translocated out of treated leaves over a period of several days. However, many species are subject to leaf abscission and much of the applied chemical is lost with the leaves. Also, the matter of absorption in xylem would tend to buffer the effectiveness of ATA. Furthermore, conditions of high transpiration are unfavorable; it is apparently a little too mobile in larger trees. However, if we consider that the applied chemical must penetrate the cuticle and pass from the apoplast into the symplast, a certain degree of mobility and lipophilic nature is essential and this, in turn, limits the translocatability of the compound. ATA, in spite of some shortcomings, would appear to embody nearly the optimum properties as a translocated herbicide. Continued use of labeled herbicides has indicated some of the pitfalls in chemical control of woody species. Aerial application often places the chemical on the wrong leaves for movement to crown and roots. Low dosage provides insufficient chemical, high dosage may cause too much contact injury. Ground application at high volume is often more successful.

Basal sprays on the bark require an oil-soluble compound and sufficient carrier for physical creeping to the crown region. This is usually an expensive treatment. Polar compounds and water as carrier meet serious obstacles to penetration through the bark. Cutting as in the cut surface treatment is inexpensive of chemical but costly of labor.

Treatment on exposed phloem as in the experiments reported here would be impractical as a field method. However, as an experimental technique it has proved worth while by providing information on the comparative behavior of the tested chemicals in the plant's vascular system. The continuous water phase in the apoplast apparently provides a ready channel for movement of chemical from phloem to xylem and hence for loss to the transpiration stream. This pinpoints the place of phloem-to-xylem transfer in the field of woody plant control. Although this phenomenon may be highly desirable in attaining systemic distribution of insecticides or fungicides in plants, it may be detrimental in the function of herbicides.

From a broader viewpoint results of experiments discussed above may offer important implications in use of insecticides, fungicides, and selective phytocides on woody plants. If such agents could be applied around the bases of trees and carried to the tops to kill predators or parasites great improvements in methods of pest control in orchards and forests might be effected.

SUMMARY

Relative mobility of 2,4-D, 2,4,5-T, ATA, MH, urea, and monuron was tested over a period of a year in the trunks of manzanita, toyon, and buckeye trees. From spot applications to the active phloem of the tree trunks there was consistent absorption of 2,4-D and 2,4,5-T by the phloem and the peak of downward movement came at the time of full development of the season's flush of growth in all three species. The other compounds were more generally carried away by the transpiration stream.

The phenoxyacetic acids were equally strongly absorbed both by the inner bark tissue and by the outer wood tissue of all three species regardless of whether they were translocated by the phloem or by the xylem.

ATA was very strongly absorbed by xylem and lightly by the inner bark

tissue of all three species regardless of whether it was transported by phloem or xylem. The translocation characteristics and mobility of the compounds were relatively consistent from species to species—however, manzanita was a better phloem translocator in general than buckeye; monuron was most mobile in manzanita; MH and urea most mobile in toyon tree trunks; and ATA in tip branches and in seedling plants of toyon.

Translocation of herbicides in the phloem can occur independently of the xylem. Downward movement in the tree trunk was in the phloem and upward movement was in the xylem.

ACKNOWLEDGMENT

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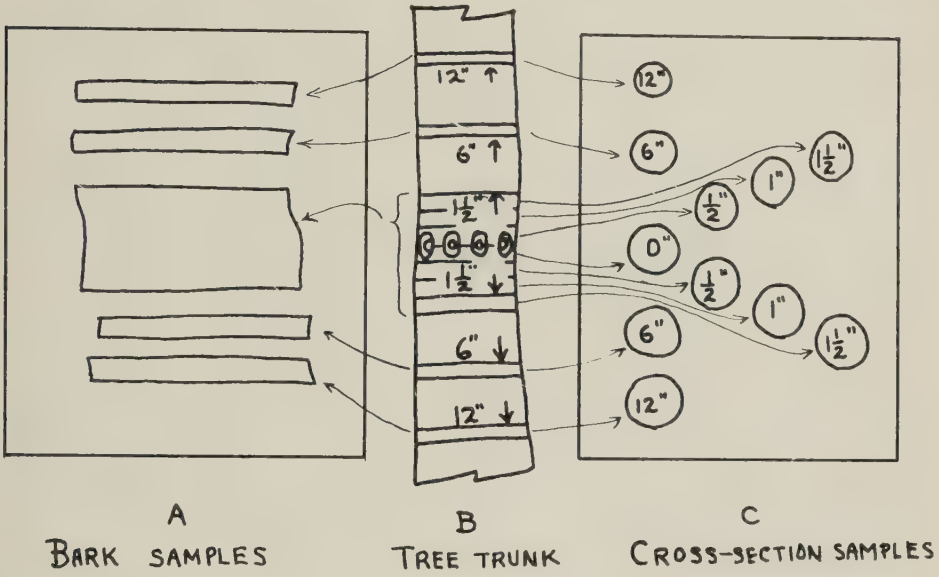

Fig. 1. Methods of sampling, illustrating (A) a mounted set of bark samples with the inner face out; (B) the tree trunk, showing the treatment level and the relative positions of the sampling; and (C) a mounted set of cross-section samples corresponding to the bark samples, showing the positions of the samples in the mount. The cross sections are ¼-inch thick, and radial orientation of the sections was maintained.

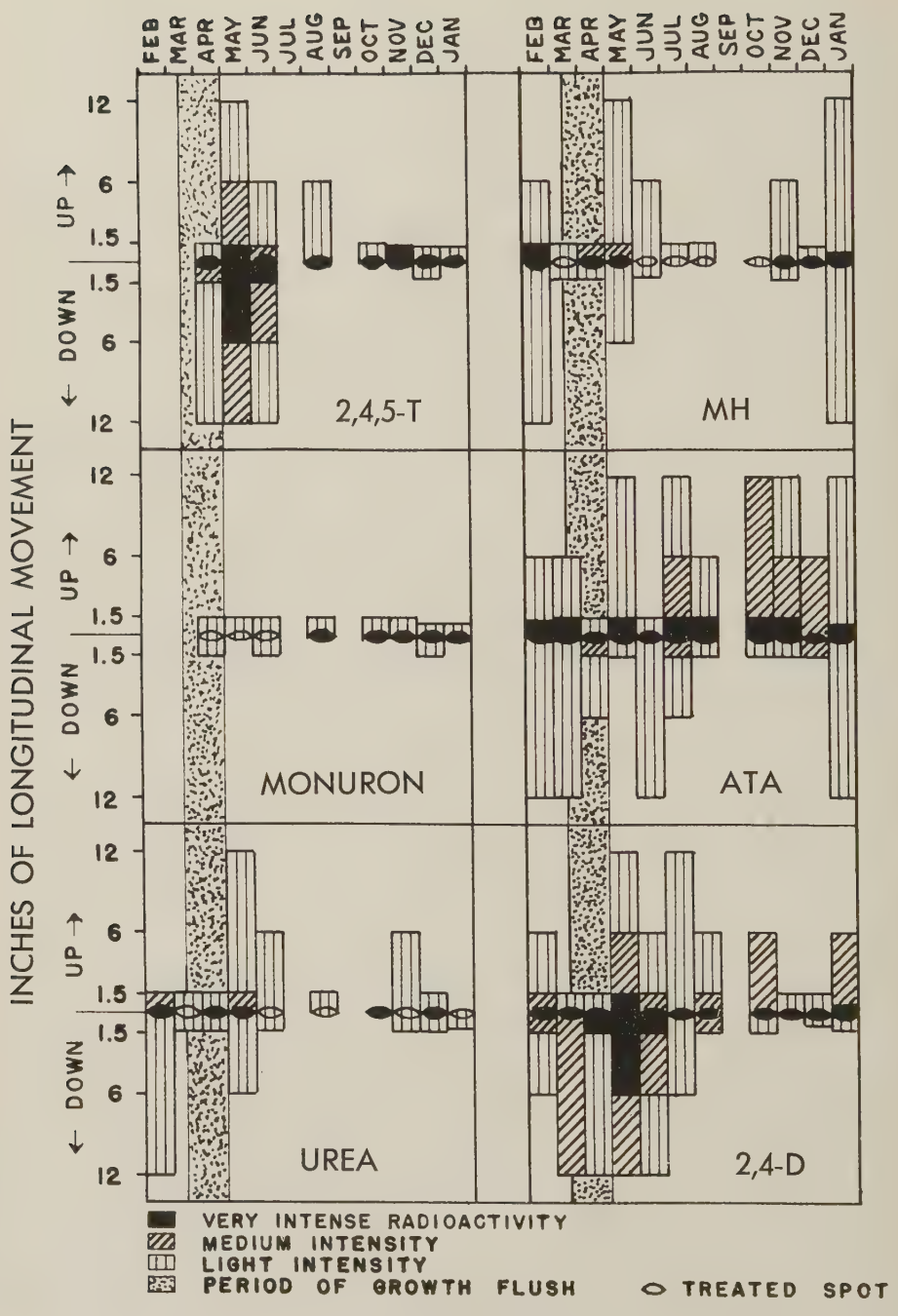

Fig. 2*a*. Manzanita. Tracing of six chemicals in the trunk.

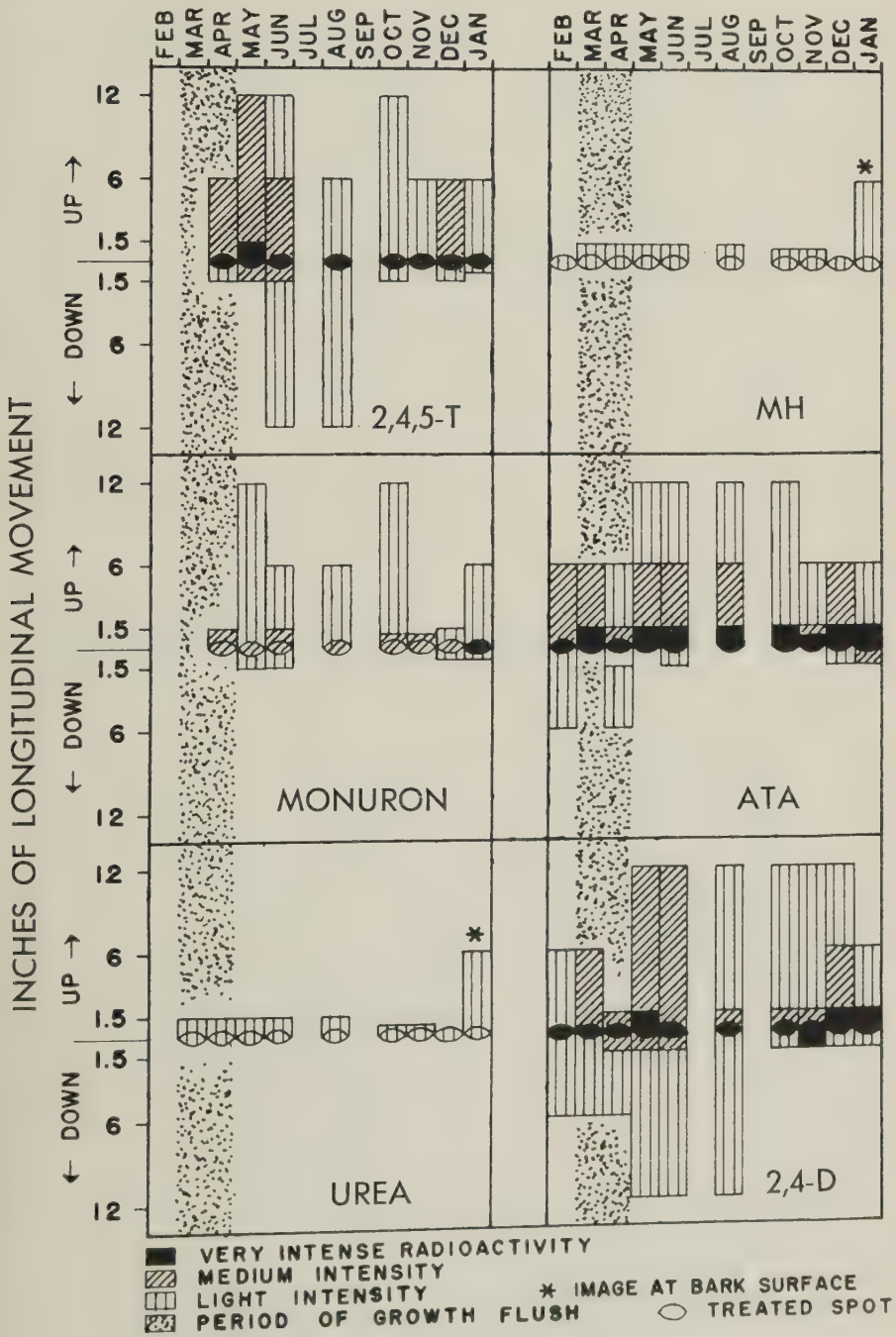

Fig. 2*b*. Toyon. Tracing of six chemicals in the trunk.

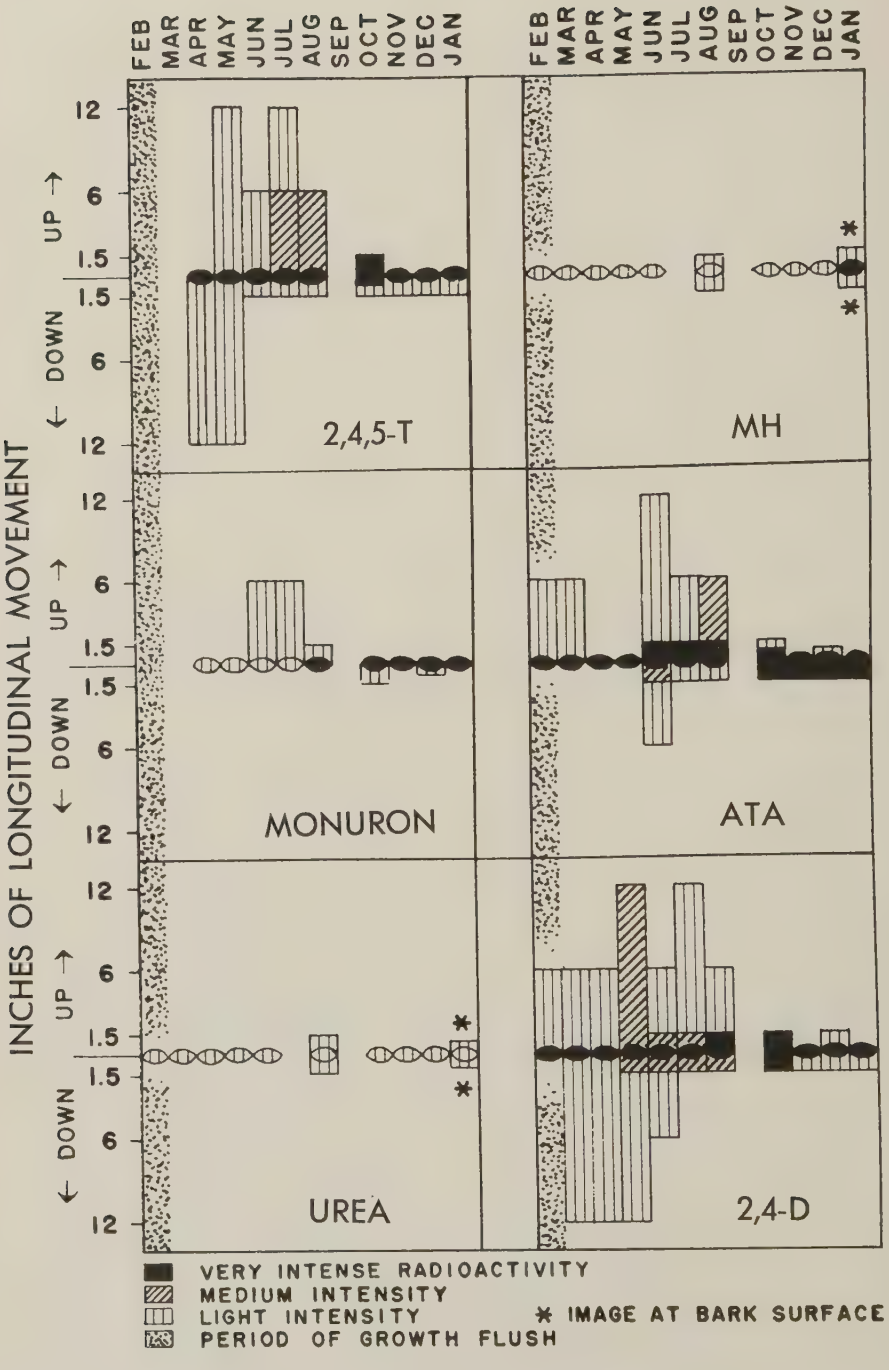

Fig. 2*c*. Buckeye. Tracing of six chemicals in the trunk.

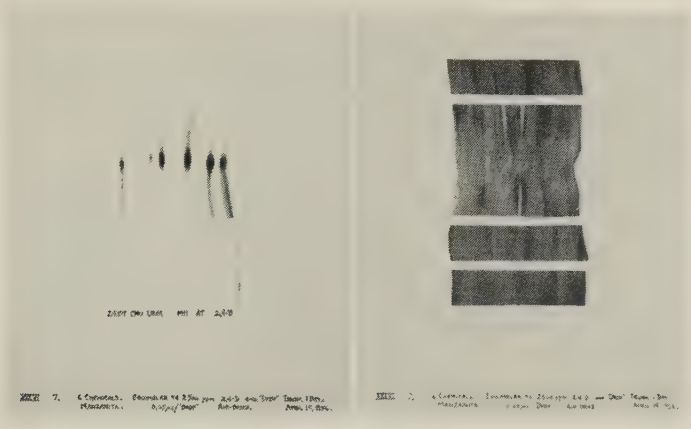

Fig. 3. Downward movement of the phenoxyacetic acids in manzanita in April. In the autograph (left) the chemicals from left to right are 2,4,5-T, monuron, urea, MH, ATA, and 2,4-D. The application spots are all very dark, except for monuron.

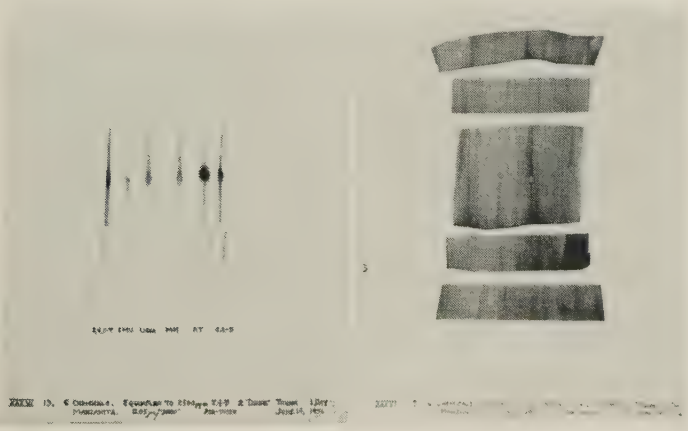

Fig. 4. Reduced downward movement and prominent upward movement of the phenoxyacetic acids in manzanita in June. In the autograph (left) the chemicals from left to right are 2,4,5-T, monuron, urea, MH, ATA, and 2,4-D.

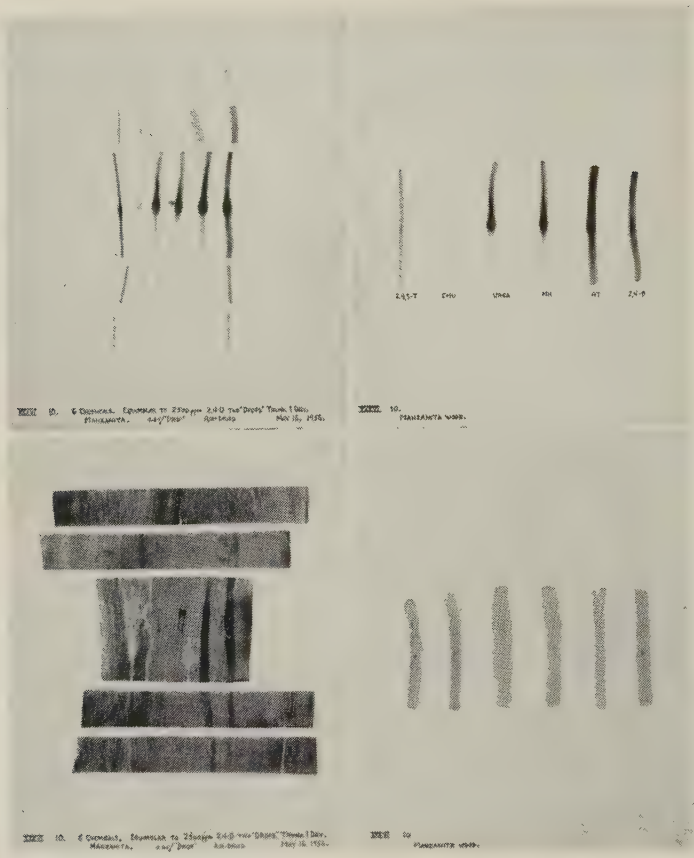

Fig. 5*a*. Manzanita, showing similarity in the amount of absorption of chemicals by phloem and by xylem, except for ATA, which was absorbed in greater quantity by xylem. Upper left is the autograph of the bark; upper right is the autograph of strips of xylem from directly underneath the treated bark. The chemicals from left to right are 2,4,5-T, monuron, urea, MH, ATA, and 2,4-D.

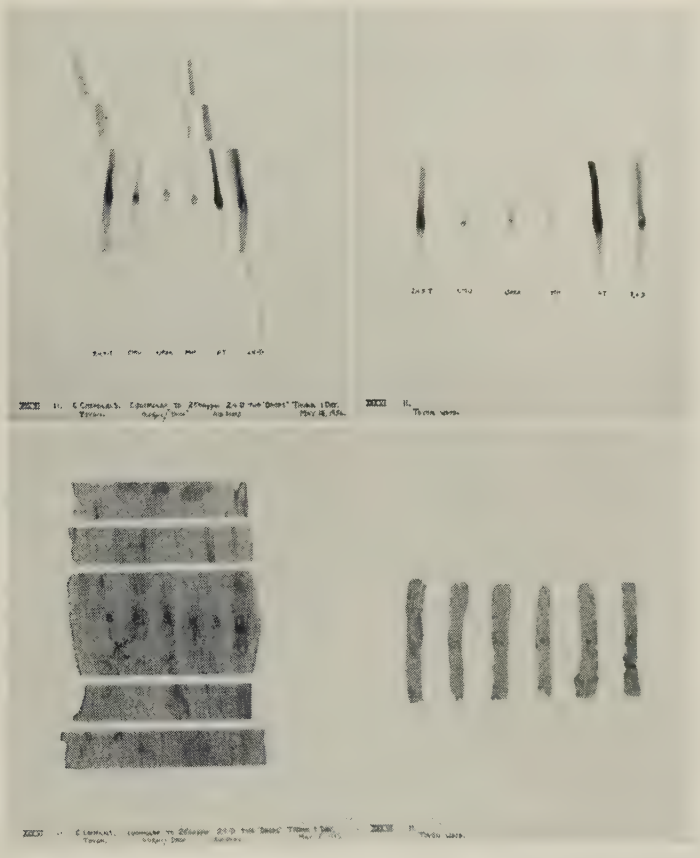

Fig. 5*b*. Toyon, showing similarity in the amount of absorption of chemicals by phloem and by xylem, except for ATA, which was absorbed in greater quantity by xylem. Upper left is the autograph of the bark; upper right is the autograph of strips of xylem from directly underneath the treated bark. The chemicals from left to right are 2,4,5-T, monuron, urea, MH, ATA, and 2,4-D.

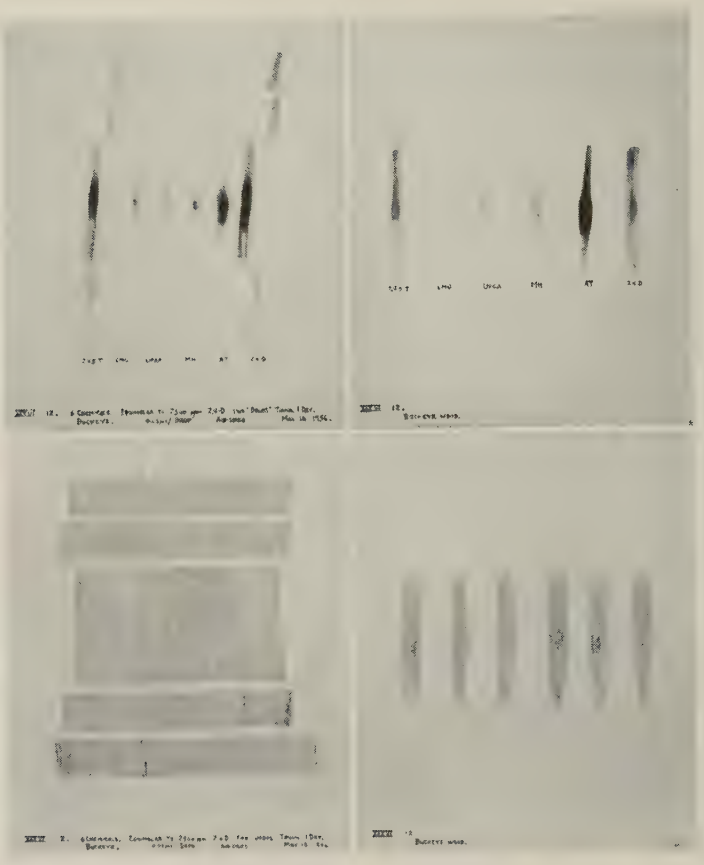

Fig. 5*c*. Buckeye, showing similarity in the amount of absorption of chemicals by phloem and by xylem, except for ATA, which was absorbed in greater quantity by xylem. Upper left is the autograph of the bark; upper right is the autograph of strips of xylem from directly underneath the treated bark. The chemicals from left to right are 2,4,5-T, monuron, urea, MH, ATA, and 2,4-D.

Fig. 6. Toyon, January treatment. The autograph of the cross sections of the treated stem indicates movement of urea and MH to the surface of the bark in the region ½ to 1½ inches above the treated level. From left to right the chemicals are 2,4,5-T, monuron, urea, MH, ATA, and 2,4-D. The arrows point to the positions of urea and MH in the cross section 1 inch above the treated level.

Fig. 7. Uptake and distribution of 2,4-D in young toyon trees. Application of the solution was made to a "cut" spot in the internode (upper left) and to an intact spot in the internode (upper right). The intense image of the very young leaves indicates a transfer of some 2,4-D from the transpiration stream to the phloem stream. Note tracing of the stem below the level of treatment.

Fig. 8. Young toyon tree showing a lack of movement of monuron into the very young leaves. The autograph is at the left.

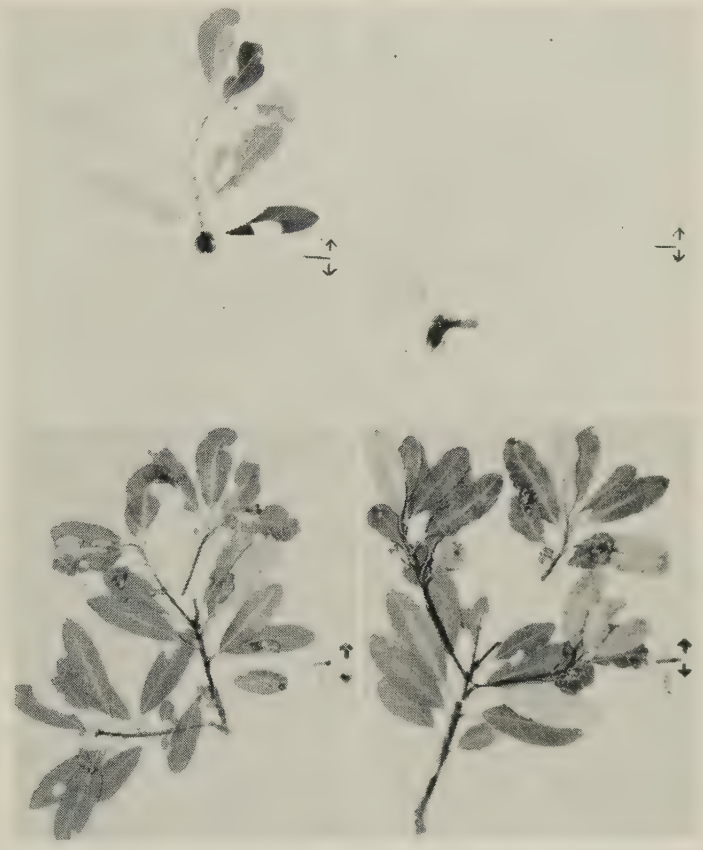

Fig. 9. September treatment of field toyon sprigs. ATA (upper left) is compared with 2,4-D (upper right). The outer bark was removed at the application spot. The dark area on the stem is the application spot. Note thorough distribution in the leaves of ATA, as compared with 2,4-D. Stem cross sections from above the 2,4-D* treated level show the presence of tracer.

Fig. 10. A small manzanita tree, treated with monuron at the base of the stem. Note intense tracing of the leaves and stem for a distance above the treated level.

Fig. 11. A small manzanita tree, treated with MH at the base of the stem. Note thorough distribution and a light tracing of the stem; also presence of MH in one root.

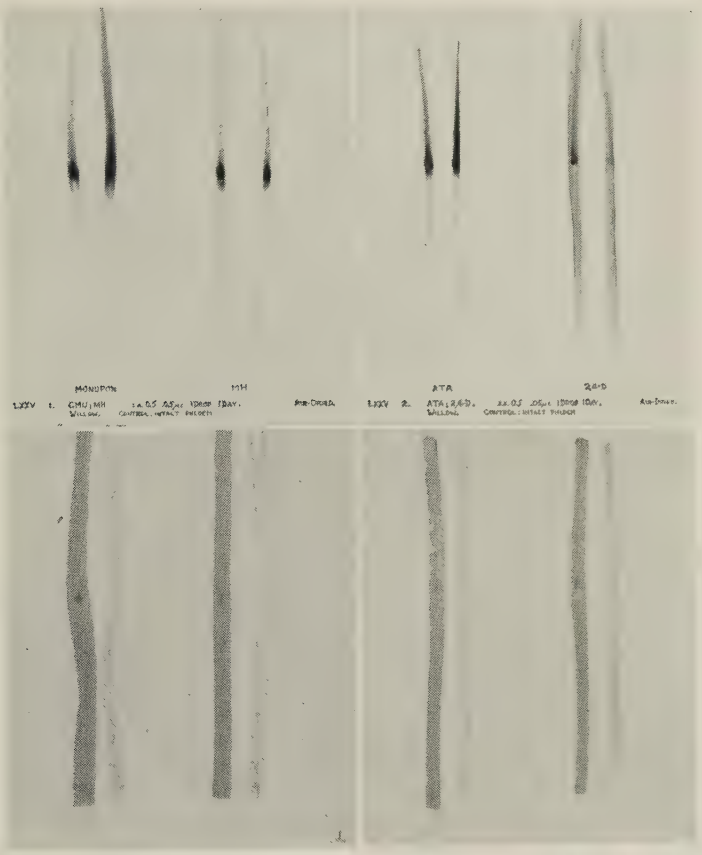

Fig. 12. Willow stem treatment with (left to right) monuron, MH, ATA, and 2,4-D. In each pair the bark is at the left and its corresponding xylem at the right. At the treatment spot the outer bark was carefully removed, leaving a quarter to a half mm of phloem tissue external to the xylem. Note downward tracing of 2,4-D in both bark and xylem.

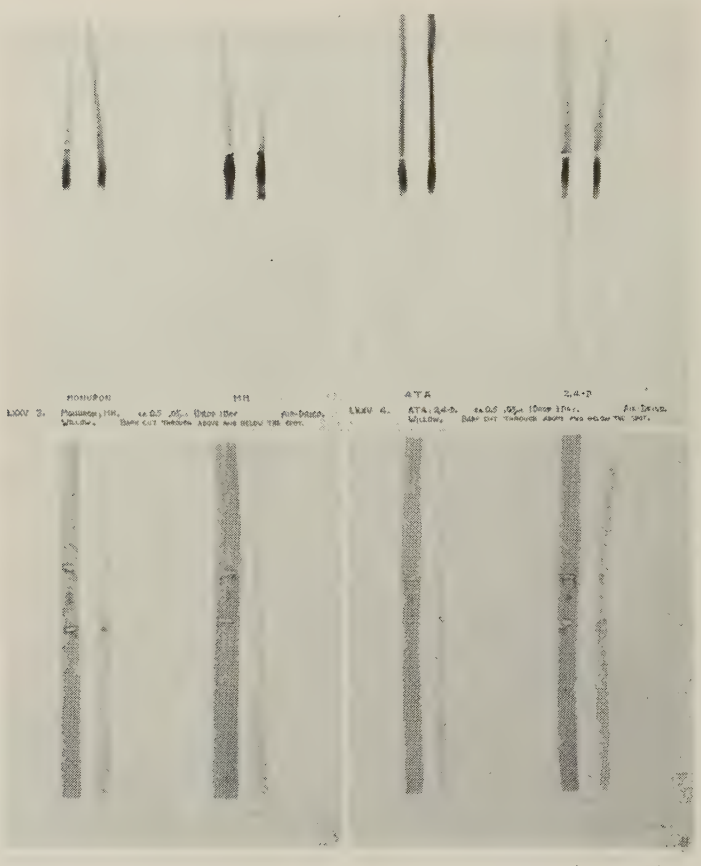

Fig. 13. Willow stems with applications made to areas of phloem tissue which were longitudinally isolated by means of transverse cuts 1 inch apart in the bark. All outer bark at the treatment spots was carefully removed. From left to right the chemicals are monuron, MH, ATA, and 2,4-D. In each pair the bark is at the left. Note the lack of downward movement of 2,4-D directly below the lower transverse cut.

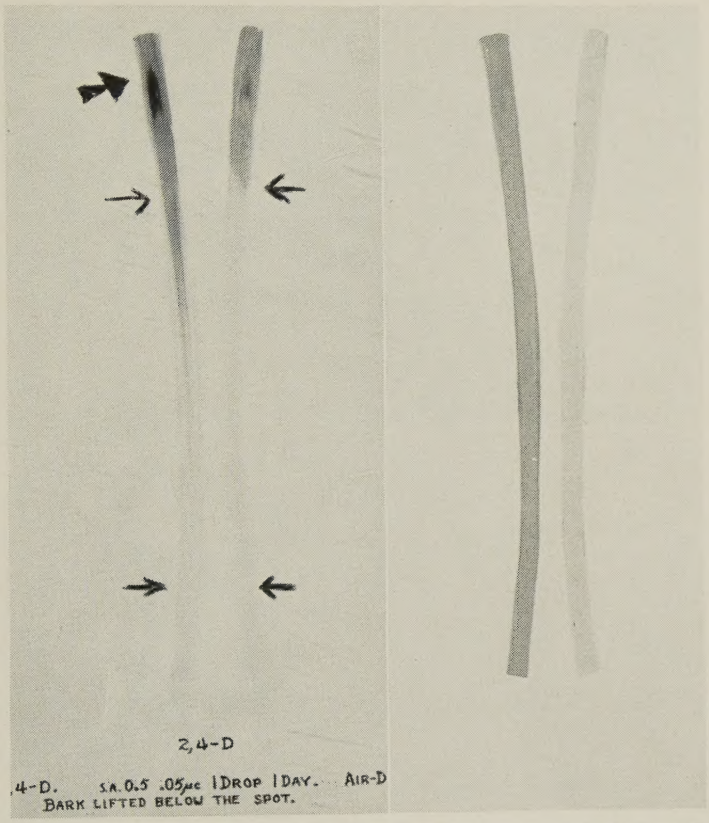

Fig. 14. Willow stem, showing the effect of lifting the bark on the downward movement of 2,4-D. A strip of bark was gently pried loose from the xylem and a piece of polyethylene was inserted. The loosened strip was taped down firmly to prevent drying. Treatment was made 1 inch above the loosened strip. In the autograph (left pair) the tracer occurs in the bark (on the left) in the loosened region. In the corresponding xylem (on the right) the tracer occurs only in the intact region. The treatment spot is indicated by the large arrow. The loosened region is between the small arrows.

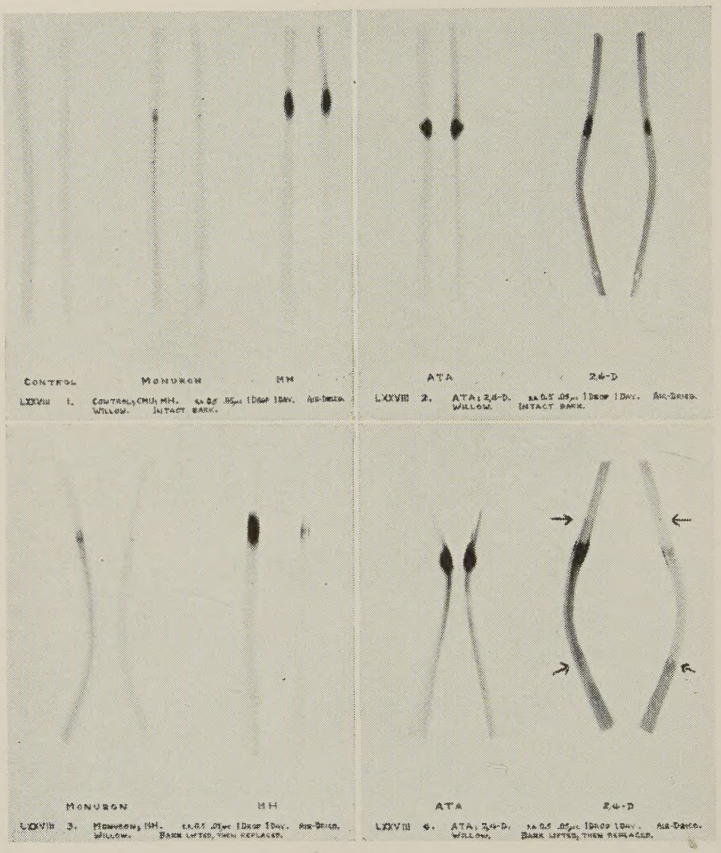

Fig. 15. Willow stem treatments, with loosened bark. Stem diameter was 1½ to 3 inches. The strip of bark (on the left) and its corresponding strip of xylem (on the right) are paired. The controls with intact bark during treatment time are in the upper row; left to right they are untreated control, monuron, MH, ATA, and 2,4-D. The experimental treatments are in the lower row; left to right, they are monuron, MH, ATA, and 2,4-D. The loosened region, about 6 inches, indicated for 2,4-D, is between the arrows. The treatment spot is near the upper end of the loosened region.